NONLACTOSE FERMENTING ORGANISMS FROM THE FECES OF INFLUENZA PATIENTS

N. P. SHERWOOD, C. M. DOWNS, AND J. B. MCNAUGHT

From the Department of Bacteriology, University of Kansas, Lawrence

Leichtenstern[1] mentions that during the pandemic of 1889-1890, at least one fourth of the cases showed no respiratory complications and were frequently confused with typhoid. Differentiation was made, however, clinically on the basis of the short incubation period, sudden onset, and comparatively short duration of the disease. Such was the condition of affairs in many parts of the United States during the pandemic of 1918. Since there seemed to be no published data showing that careful laboratory methods had been used to differentiate mild typhoid and paratyphoid—enteritidis infections from influenza, it seemed worth while to make bacteriologic examinations of feces in recurring epidemics. This seemed more warranted after looking over the reports of the Kansas state epidemiologist, which showed a marked drop over previous years in reported cases of typhoid and paratyphoid fever.

The first cases studied were a few of typical influenzal pneumonia at Lawrence, Kan., during Dec., 1918. Relatively large recurring epidemics occurred at Wichita, in February and at Topeka, McPherson and other points in Kansas during March and April. Fecal examinations and in many instances blood cultures, blood counts and agglutination tests were made on typical cases in the isolation hospitals of these places. A series of control cases was studied at Topeka and also at Lawrence.

The technic of the fecal examination was: Samples were obtained as early as possible in the disease and wherever possible repeated examinations were made. In cases of constipation, the first fecal masses were discarded, and the more fluid portion containing some mucus was used for plating. Eosin-methylene blue agar plates, as suggested by Holt-Harris and Teague[2] were used. Four plates for undiluted feces and four plates for broth dilution were streaked. After 24 hours' incubation, colonies were fished and were customarily restreaked on eosin-blue plates and from these cultures were obtained for study. At first, several colonies of each type were picked but our experience at Lawrence and Wichita caused us to pick, if possible, a minimum of 15 colonies from any resembling typhoid or paratyphoid types and 2 or 3 of each of the other kinds. Suspicious colonies of the former types were, after replating, inoculated in new Russell's medium, morphology studied and

Received for publication Aug. 28, 1919.

[1] Nothnagels Encylopedia, Practical Medicine, 1905, p. 591.
[2] Jour. Infect. Dis., 1916, 18, p. 596.

Gram's stains made, and then tested out if necessary in litmus milk, gelatin and the following sugars and alcohols: dextrose, mannite, lactose, saccharose, arabinose, dulcite, xylose, rhamnose and salacin. New York Board of Health and American Museum strains of paratyphoid A, paratyphoid B, enteritidis and typhoid were used as control organisms. In addition, blood from a number of convalescent patients was obtained for agglutination tests. None of these patients had been vaccinated against any of these groups.

A summary of the results is shown in table 1.

TABLE 1

SUMMARY OF RESULTS

Source	Number of Samples from Typical Cases	Number of Samples from Control Cases	Kind of Organisms Found in Feces and Number of Samples Showing Same			Percentages of Cases Showing	
			Morgan's Bacillus	Enteritidis-like Organisms	B. typhosus	Typhoid or Enteritidis-like Organisms	Enteritidis-like Organisms
Isolation Hospital, Wichita	9	..	1	3	1	44%	33%
Isolation Hospital, Topeka	15	..	2	10	1	73%	66%
Noninfluenza cases, Topeka	..	12	None	None	None	None	None
Private cases, McPherson...	2	..	None	1	None	50%	50%
Private cases, Lawrence.....	6	..	None	3	1	66%	50%
Noninfluenzal private cases, Lawrence.....	..	10	None	None	1	10%	None

From table 1 it will be observed that at Wichita the feces from 9 cases were examined and enteritidis-like organisms found in 3, and B. typhosus in a fourth case. Morgan's bacillus was also found in one case associated with enteritidis-like organisms.

In the Topeka epidemic the feces from 15 cases were examined with the result that 10 were positive for enteritidis-like organisms and 1 for B. typhosus. Morgan's bacillus was found associated with other enteritidis-like organisms in 2 of the 10 positive cases. The 12 noninfluenza control cases were negative.

At Lawrence, enteritidis-like organisms were obtained from 3 of 6, and B. typhosus from a fourth case of influenza. None were obtained from 10 control cases, such as mumps, abscesses, 1 of typical typhoid fever, 2 cases of measles, 3 cases of tonsillitis and several cases of lobar pneumonia. Neither Morgan' bacillus nor paracolon bacilli were found.

The 2 cases at McPherson were mild ones. Enteritidis-like organisms were obtained from 1 of the 2 cases in children under 12 years of age. At McPherson a large epidemic was just ending when the work was done.

In view of the fact that enteritidis-like organisms were isolated in 33-⅓% of the cases at Wichita, 66% of those at Topeka, 50% of those at Lawrence, and 50% of those at McPherson, Kansas, most of them widely separated points, it seems worth while to state the cultural characteristics of these strains (table 2).

According to Jordan's[3] studies on this group, the organisms we have described would be classified as B. paratyphosus B, or B. enteritidis, showing some variation in dulcite. Strain 12 is atypical in xylose, while strain 3 gave no acid or gas in rhamnose. In regard to strain 10 which ferments salicin, it is of interest that a second sample of feces from this case gave us a number of salicin negative strains. Strain 11 showed some gas after 36 hours.

Krumwiede, Kohn and Valentine[4] observed variation in dulcite fermentation in their studies on the paratyphoid enteritidis group and summarize their findings as follows: "Evidently no one characteristic has served to differentiate between all strains of the B. suis and B. paratyphosus 'B' strains. Dulcite fermentation, as with most other members of the group, varies according to sub-group avidity, and has differential value only in so far as a low avidity indicates that a strain does not belong to the 'B' group."

It will be noted further from table 2 that all of our strains blackened lead acetate agar. According to Jordan,[5] B. suipestifer and B. paratyphosus A, consistently fail to do this, while B. paratyphosus B, and B. enteritidis uniformly blacken this medium. Since table 2 does not show the length of time the litmus milk cultures were kept under observation, it may be of interest to note that this was for a period of 3 weeks. Alkalinity persisted and no saponification occurred.

After we had obtained a number of strains at Topeka, it was decided to make agglutination tests using blood from convalescent patients and several strains from both Wichita and Topeka. The results were not at all consistent. Some tests were entirely negative with all strains, some negative with their own strains and positive with others, while some were positive with their own strains as well as a few of the others. A 1:60 dilution of serum was used. It was decided to check up the relationship by agglutination and absorption tests.

[3] Jour. Infect. Dis., 1917, 20, p. 457.
[4] Jour. Med. Research, 1918, 38, p. 89.
[5] Jour. Infect. Dis., 1917, 21, p. 554.

TABLE 2

GRAM-NEGATIVE, NONLACTOSE FERMENTING BACILLI ISOLATED FROM SUPPOSEDLY TRUE INFLUENZA CASES

City	Organism Number	Gelatin Liquefaction	Motility	Litmus Milk				Fermenting Acid and Gas, 24 Hours									Blackening of Lead Acetate Agar
				24 Hours	48 Hours	72 Hours	5 Days	Dextrose	Mannite	Saccharose	Arabinose	Dulcite 30 Hours	Xylose	Rhamnose	Salicin	Indol	
Wichita	1	—	+	A	al	al	al	⊕	⊕	—	⊕	—	⊕	⊕	—	—	+
	2	—	+	A	al	al	al	⊕	⊕	—	⊕	⊕	⊕	⊕			
	3	—	+	A	al	al	al	⊕	⊕	—	⊕	—	⊕	—	—	—	+
Topeka	4	—	+	A	al	al	al	⊕	⊕	—	⊕	—	⊕	⊕	—	—	+
	5	—	+	A	al	al	al	⊕	⊕	—	⊕	—	⊕	⊕	—	—	+
	6	—	+	A	al	al	al	⊕	⊕	—	⊕	—	⊕	⊕	—	—	+
	7	—	+	A	al	al	al	⊕	⊕	—	⊕	—	⊕	⊕	—	—	+
	8	—	+	A	al	al	al	⊕	⊕	—	⊕	⊕	⊕	⊕	—	—	+
	9	—	+	A	al	al	al	⊕	⊕	—	⊕	—	⊕	⊕	—	—	+
	10	—	+	A	al	al	al	⊕	⊕	—	⊕	⊕	⊕	⊕	⊕	—	+
	11	—	+	A	al	al	al	⊕	⊕	—	⊕	⊕	⊕	⊕	—	—	+
	12	—	+	A	al	al	al	⊕	⊕	—	⊕	—	—	—	—	—	+
	13	—	+	A	al	al	al	⊕	⊕	—	⊕	⊕	⊕	⊕	—	—	+
Lawrence	14	—	+	A	al	al	al	⊕	⊕	—	⊕	⊕	⊕	⊕	—	—	+
	15	—	+	A	al	al	al	⊕	⊕	—	⊕	⊕	⊕	⊕	—	—	+
	16	—	+	A	al	al	al	⊕	⊕	—	⊕	⊕	⊕	⊕	—	—	+
McPherson	17	..	+	A	al	al	al	⊕	⊕	—	⊕	⊕	⊕	⊕	—	—	+
Chicago University	B. suipestifer	—	+	A	A	A	al	⊕	⊕	—	—	—	⊕	⊕	..	..	—
New York Health Laboratory Strains of	Paratyphoid A	—	+	A	A	A	A	⊕	⊕	—	⊕	—	—	⊕	—	—	—
	Paratyphoid B	—	+	A	A	al	al	⊕	⊕	—	⊕	⊕	⊕	⊕	—	—	+
	B. Enteritidis	—	+	A	A	al	al	⊕	⊕	—	⊕	⊕	⊕	⊕	—	—	+

The results of cross agglutination and absorption tests may be summarized as follows:

Rabbits were tested for normal agglutinins and then immunized and specific immune serum for each of the strains isolated was obtained. In addition, immune serum was produced for our laboratory strains of B. enteritidis, B. paratyphosus A and B, and for two strains of B. suipestifer. Cultures of B. typhimurium and B. cavipesticus were obtained from the New York City Health Laboratory. Other strains of members of this group were supplied by the University of Chicago and by the Iowa State Agricultural College. In addition, immune serums for paratyphoid A and B were obtained from one of the commercial supply houses.

The strains of enteritidis-like organisms isolated from influenza cases showed practically the same slight amount of cross agglutination with paratyphoid B immune serum as the known strains of B. enteritidis we used. None showed any relationship to paratyphoid B by absorption tests. None showed any relationship by absorption tests to B. suipestifer, typhimurium, cavipesticus or paratyphoid A.

Many of them showed decided cross-agglutination with each other and with B. enteritidis. The absorption tests apparently divided them into the groups shown in table 3.

TABLE 3

SHOWING POSSIBLE TYPING OF ENTERITIDIS GROUP BY AGGLUTINATION AND ABSORPTION TESTS

	Classification of Strains According to Type				
	1 B. enteritidis	2	3	3 Atypical	4 Heterogeneous Group
	13	7 9	5 6 10 12 16 17	3 14	1 2 4 8 11
Total in each type*..	1	2	6	2	5

* Strain Number 15 was lost before typing was done.

It will be observed from table 3 that 1 strain was found to be identical with our laboratory strain of B. enteritidis, 2 strains fell into type 2, and 6 strains into type 3, and 2 into atypical type 3. By absorption the atypical type 3 strains reduced the titre of type 3 immune serum from 1-8000 to 1-160. These 3 types are well marked and specific groups; type 4 is a heterogeneous group the members of which show very little if any relationship with each other by absorption tests, and each member is agglutinated strongly by its serum only. This is

analogous to the type of pneumococci. It may well be that with a larger series of organisms, the division might be extended to include one or more additional specific types.

There was no apparent correlation between the presence of these organisms and the type of stools, e. g., strain 13 in type 1 was apparently a case of influenzal pneumonia of the constipated type.

The 2 cases in type 2 were mild uncomplicated cases of influenza with constipation.

The 8 strains falling in type 3 were associated with cases as follows: Strains 5, 6, 10 and 12 were from mild, constipated cases of influenza, while strain 16 was from a severe case associated with diarrhea without pneumonia and strains 17 and 3 from influenzal-pneumonias of the diarrheal type. Strain 14 was from a case of constipated influenzal-pneumonia.

In the heterogeneous group, or type 4, strains 1 and 8 were associated with influenzal pneumonia, one case being of the diarrheal type and the other of the constipated; strain 2 came from an uncomplicated influenza with diarrhea, while 4 and 11 came from uncomplicated influenza, with constipation.

SUMMARY AND CONCLUSIONS

Bacteriologic examinations of the feces of 32 patients with influenza showed the presence of B. typhosus in 3 and of enteritidis-like organisms in 17 others. Examination in control cases (1 case of typhoid and 1 of mumps, 2 of measles, 3 of tonsillitis, several of lobar pneumonia and several surgical cases) yielded B. typhosus in the case of typhoid, but no enteritidis-like organisms in any of the other cases.

The presence of B. typhosus in three of the cases of influenza may be interpreted as influenza cases among typhoid carriers or mild cases of typhoid resembling influenza.

The significance of the enteritidis-like organisms is unknown.

The 17 strains of enteritidis-like organisms would be classified according to Jordan as either B. paratyphosus B, or B. enteritidis, with several of the strains atypical in dulcite. The relationship established by absorption tests shows that dulcite fermentation is variable in apparently identical strains.

By agglutination and absorption tests, these strains seem to show no relationship to B. paratyphosus A or B, B. suipestifer, B. typhimurium and B. cavipesticus. Many of them show relationship to our

laboratory strain of B. enteritidis and one strain was found to be identical with it. This suggests that B. enteritidis may be typed similarly to the pneumococci. Our strains seemed to fall into 4 types, 3 specific and 1 heterogeneous group. We feel that this is more rational than calling them new organisms.

There seemed to be no correlation between the nature of stools and the presence of these organisms. They were found in both diarrheal and constipated stools, in mild and severe cases of influenza.

If these organisms have no clinical significance in these cases, it would seem to raise the question as to the value of much of the published work on epidemics of food poisoning, supposedly due to enteritidis-like organisms where the mere presence in feces coupled with more or less vague clinical pictures quite similar to many cases of influenza have been assumed to prove the enteritidis-like organisms as the causative factor.

Our results would seem to warrant more work being done on the gastro-intestinal tract in influenza.